has so far been unsatisfactory. Fractional crystallization leaves the active substance in a syrupy filtrate. Precipitation with $AgSO_4 + Ba(OH)_2$, of histidine and a syrupy material giving the histidine diazo test, leaves most of the active material in the filtrate. The addition of an excess of baryta causes some, but not all, to be thrown down together with impurities as the silver compound. Phosphotungstic acid apparently destroys the activity of the compound, although this requires further verification. It has not been possible to obtain active material either from the phosphotungstic precipitate or filtrate. The phosphotungstic acid has been removed both by baryta and by extracting with amyl alcohol and ether.

In attempting other methods, also, the activity has gradually diminished and been entirely lost, and it may prove impossible to obtain the material in pure form by methods at present available. It is hoped, however, that further work with larger quantities of material will result in the separation of this compound, which may prove to be of more general interest than simply from the standpoint of bacterial nutrition.

To sum up: the experiments here reported indicate that casein and certain other proteins contain a hitherto undescribed component, which also occurs in an infusion of beef and beef heart. It is essential to the growth of the hemolytic streptococcus and probably the pneumococcus, and is absorbed from the beef infusion by charcoal, and precipitated from the casein in an impure form by mercuric sulphate. The chemical nature of the substance has not yet been determined.

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The cause of the parallelism between the gram reaction and the gentian violet reaction.

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In previous studies, published at intervals since 1912, it has been shown that a striking parallelism exists between the Gram

reaction and the gentian violet reaction. The Gram positive organisms are killed by the stain and will not grow in agar containing it; the Gram negative organisms survive staining and grow vigorously in the presence of the dye. To this rule there are about 10 per cent. of exceptions.

Does this parallelism indicate that the two reactions have fundamentally the same explanation and that the power of the Gram positive organisms to fix the dye, so that it is retained in the Gram process, enables them also to fix it so that it leads to their death, or prevents their growth in media containing it? An attempt was made to answer this question by training a Gram positive organism (*B. subtilis*) to grow on agar containing gentian violet, working up gradually from minimal dilutions (1–1,000,000) to greater strengths. If a Gram positive organism so trained ceased to retain the stain by Gram's method the problem would be solved. This attempt was, however, wholly unsuccessful; it was impossible to train *B. subtilis* to grow in the presence of the dye.

A study of a Gram negative organism (*B. coli*)—which is also gentian negative—gave a partial answer to the question. If thick suspensions of this organism be stroked across a divided gentian violet plate, growth is equally vigorous on the two sides; the organism is apparently in no way restrained by the dye. If, however, instead of a thick suspension increasingly weak dilutions of the suspension be used for the stroking, the colonies on the gentian violet side of the plate become rapidly fewer as the dilution increases, and soon disappear altogether. The same result was obtained by similar experiments with other Gram negatives (*B. typhosus* and *B. prodigiosus*). That is to say: *In a thick suspension of a Gram negative organism only a small proportion of the individuals are Gentian negative; it is possible to isolate the Gentian positive individuals in pure culture, and when so isolated they are found to be as definitely Gram negative as the Gentian negative individuals. The factor which determines the reaction of an organism to the Gram process of staining is therefore not the same as the factor which determines its reaction on divided gentian violet plates, or after staining with the dye.*